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Inhibitory effect of anti-diabetic agents on rat organic anion transporter rOAT1

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Abstract

The interactions of sulfonylureas and a novel anti-diabetic drug, nateglinide, with rat renal organic anion transporter (rOAT1) expressed in *Xenopus laevis* oocytes were studied. Uptake of *p*-aminohippurate via rOAT1 was markedly inhibited by glibenclamide and nateglinide, and moderately by chlorpropamide and tolbutamide. The inhibition constant values (K_i) for chlorpropamide, glibenclamide, tolbutamide and nateglinide were 39.5, 1.6, 55.5 and 9.2 μM, respectively. Kinetic analysis showed that the inhibition of *p*-aminohippurate uptake by glibenclamide was competitive. Sulfonylureas examined and nateglinide did not show a trans-stimulation effect on [^{14}C] *p*-aminohippurate efflux from rOAT1-expressing oocytes. There was no stimulation of [^{3}H]glibenclamide uptake via rOAT1. These findings suggested that sulfonylureas and nateglinide interact with rOAT1, but these drugs are not translocated via the transporter.

Keywords: OAT1 (organic anion transporter 1); Renal tubular secretion; *Xenopus* oocytes; Sulfonylurea; Nateglinide; *p*-Aminohippurate

1. Introduction

Renal handling of structurally diverse drugs and xenobiotics exerts pharmacological effects as well as adverse toxic effects. In renal proximal tubules, organic anion and cation transport systems contribute to renal tubular secretion of negatively and positively charged compounds, respectively (Inui and Okuda, 1998; Pritchard and Miller, 1993). The renal organic anion transport system has been well characterized using isolated renal tubules (Chatsudthipong and Dantzler, 1992; Miller and Pritchard, 1991; Schäli and Roch-Ramel, 1980), isolated membrane vesicles from the renal cortex (Inui et al., 1986; Martinez et al., 1990; Shimada et al., 1987) and cultured renal cells such as opossum kidney cells (Nagai et al., 1995; Takano et al., 1996). These studies suggested that renal tubular secretion of a prototypical organic anion, *p*-aminohippurate, was performed by two distinct transport processes: the first cellular uptake across the basolateral membranes of tubular cells is driven by exchange with α-ketoglutarate, and the second step of efflux out of the tubular cells across the brush-border membranes into the lumen depends on the transmembrane potential difference and on an organic anion/anion exchange.

Recently, several cDNA clones encoding organic anion transporter proteins belonging to the organic anion transporter gene family in the rat kidney have been identified and designated as OAT1/ROAT1, OAT2 and OAT3 (Sekine et al., 1997, 1998; Sweet et al., 1997; Kusuhara et al., 1999). When expressed in *Xenopus* oocytes, they showed *p*-aminohippurate uptake and broad substrate specificities (Kusuhara et al., 1999; Sekine et al., 1997, 1998; Sweet et al., 1997; Uwai et al., 1998). Of these transporters, rOAT1 has been characterized as the predominant *p*-aminohippurate transporter (*p*-aminohippurate/α-ketoglutarate exchanger) in the renal basolateral membranes (Sekine et al., 1997; Sweet et al., 1997; Uwai et al., 1998).

Sulfonylureas such as chlorpropamide, glibenclamide and tolbutamide are administered to patients with Type 2 diabetes (non-insulin-dependent diabetes mellitus), which show their pharmacological effect by induction of insulin secretion due to blocking ATP-sensitive K^+ channels in pancreatic β-cells (Babenko et al., 1998). There are many combined drugs that induce the hypoglycemia side effect by increasing plasma concentration of sulfonylureas in clinical situations (Hansen and Christensen, 1977). Probenecid, a potent organic anion transport system in-

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hibitor is included among these drugs. The suggested mechanism of the interaction is that the renal secretion of sulfonylureas by the organic anion transport system could be suppressed by probenecid (Petitpierre et al., 1972).

A novel oral hypoglycemic agent nateglinide is a non-sulfonylurea anti-diabetic agent, which stimulates insulin release through the same mechanism as sulfonylureas, although the agent shows a more rapid and briefer decrease in blood glucose than sulfonylureas (Ikenoue et al., 1997). The unique feature of nateglinide is rapid intestinal absorption and renal elimination. There are few reports, however, demonstrating the pharmacokinetics including the renal handling of the drug as well as interaction with tubular transport systems.

In the present study, we examined the interaction of sulfonylureas and nateglinide with rOAT1 using the *Xenopus* oocyte expression system. The findings suggest that these drugs interact with rOAT1, but are not transported by the transporter.

2. Materials and methods

2.1. Materials

[Glycyl-^{14}C] *p*-aminohippurate (1.9 GBq/mmol) and [cyclohexyl-2,3-^{3}H(N)]-glibenclamide (1.85 TBq/mmol) were purchased from Du Pont-New England Nuclear Research Product (Boston, MA, USA). Chlorpropamide and tolbutamide were obtained from Sigma (St. Louis, MO, USA). Glibenclamide was from Wako (Osaka, Japan). A new oral anti-diabetic drug nateglinide was from Ajinomoto (Yokohama, Japan). All other chemicals used were of the highest purity available.

2.2. Functional expression of rOAT1 in Xenopus oocytes

Capped cRNA of rOAT1 was transcribed from *Not*I-linearized pSPORT containing rOAT1 cDNA with T7 RNA polymerase as described (Uwai et al., 1998). After *Xenopus* oocytes were injected with 50 nl of water or rOAT1 cRNA (25 ng), the oocytes were maintained in modified Barth's medium (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.4 mM $CaCl_2$, 0.8 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 10 mM HEPES) containing 50 μg/ml gentamicin at 18°C.

2.3. Uptake reaction

Two days after injection, the uptake study was initiated by incubating oocytes in 500 μl of uptake buffer (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$ and 5 mM HEPES; pH 7.4) containing [^{14}C] *p*-aminohippurate or [^{3}H]glibenclamide at 25°C in the presence or absence of inhibitors for 1 h except otherwise indicated. Chlorpropamide, tolbutamide and nateglinide were dissolved in acetonitrile whose final concentration in uptake buffer was less than 0.5%. Glibenclamide was dissolved in dimethyl sulphoxide (DMSO), and its final concentration in uptake buffer was less than 1%. The uptake reaction was terminated by adding 2 ml of ice-cold uptake buffer to each well, and the oocytes were washed five times with 2 ml of the buffer. After washing, each oocyte was transferred to a single vial and dissolved with 500 μl of 10% sodium lauryl sulfate (SDS). The radioactivity was determined by adding 5 ml of ACSII (Amersham International, UK) to each solubilized oocyte in a liquid scintillation counter.

2.4. Efflux experiment of [^{14}C]p-aminohippurate

For the efflux measurement, oocytes injected with rOAT1 cRNA were incubated in the uptake buffer containing 50 μM [^{14}C] *p*-aminohippurate for 2 h at 25°C. After uptake, the oocytes were washed five times with 2 ml of ice-cold uptake buffer and transferred to 1.5-ml tubes containing 500 μl of the uptake buffer containing the compounds to be tested, and incubated for 90 min at 25°C. The radioactivity associated with both the oocytes and the incubation medium was counted for calculation of the efflux of [^{14}C] *p*-aminohippurate out of the oocytes.

3. Results

3.1. Effects of sulfonylureas and nateglinide on p-aminohippurate uptake by rOAT1-expressing oocytes

As shown in Fig. 1, uptake of [^{14}C] *p*-aminohippurate was linearly increased up to 1 h in *Xenopus* oocytes

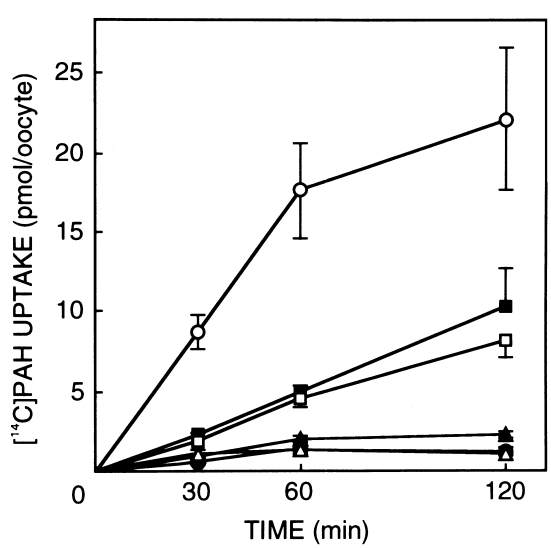

Fig. 1. Effect of antidiabetic drugs on [^{14}C] *p*-aminohippurate uptake by oocytes injected with water or rOAT1 cRNA. Uptake of [^{14}C] *p*-aminohippurate (25 μM) by water (●) or rOAT1 cRNA injected oocytes in the absence (○) or presence of 1 mM chlorpropamide (□), 50 μM glibenclamide (▲), 1 mM tolbutamide (■) or 1 mM nateglinide (△). Each point represents the mean ± S.E. of 7–10 oocytes.

injected with rOAT1 cRNA. The *p*-aminohippurate uptake by rOAT1 was moderately inhibited in the presence of 1 mM chlorpropamide and tolbutamide, and strongly in 50 μM glibenclamide and 1 mM nateglinide. However, 0.5% acetonitrile and 1% DMSO did not affect rOAT1-mediated uptake of *p*-aminohippurate (data not shown).

3.2. Dose-dependent inhibition of rOAT1-mediated p-aminohippurate uptake by sulfonylureas and nateglinide

To compare the inhibitory potencies of chlorpropamide, glibenclamide, tolbutamide and nateglinide, we examined the dose dependence of the inhibition (Fig. 2). The *p*-aminohippurate uptake by rOAT1 was inhibited by glibenclamide, nateglinide, chlorpropamide and tolbutamide in the order of inhibitory potency. The estimated IC_{50} values for the *p*-aminohippurate uptake were 4.0 μM for glibenclamide, 22.8 μM for nateglinide, 97.6 μM for chlorpropamide and 137.1 μM for tolbutamide.

3.3. Inhibition style of glibenclamide on rOAT1-mediated p-aminohippurate uptake

To determine the inhibition mode of glibenclamide, dose-dependent *p*-aminohippurate uptake by rOAT1 in the presence of 10 μM glibenclamide was performed. As shown in Fig. 3, glibenclamide decreased the affinity of *p*-aminohippurate for rOAT1, with the apparent Michaelis–Menten constant value (K_m) being increased from 18 to 409 μM, whereas the maximum uptake rate (V_{max}) did not change. These findings and the Eadie–Hofstee plots suggested that glibenclamide inhibits rOAT1-mediated *p*-aminohippurate transport in a competitive manner.

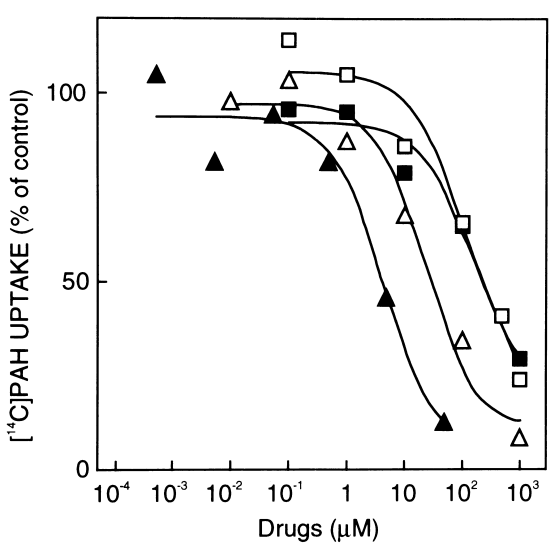

Fig. 2. Dose dependence of inhibition of *p*-aminohippurate uptake by various antidiabetic drugs in rOAT1-expressing oocytes. rOAT1-mediated [^{14}C]*p*-aminohippurate uptake (25 μM) for 1 h was determined in the absence or presence of chlorpropamide (□), glibenclamide (▲), tolbutamide (■) or nateglinide (△). Each point represents the mean of two experiments. Each experiment was performed using 5–10 oocytes.

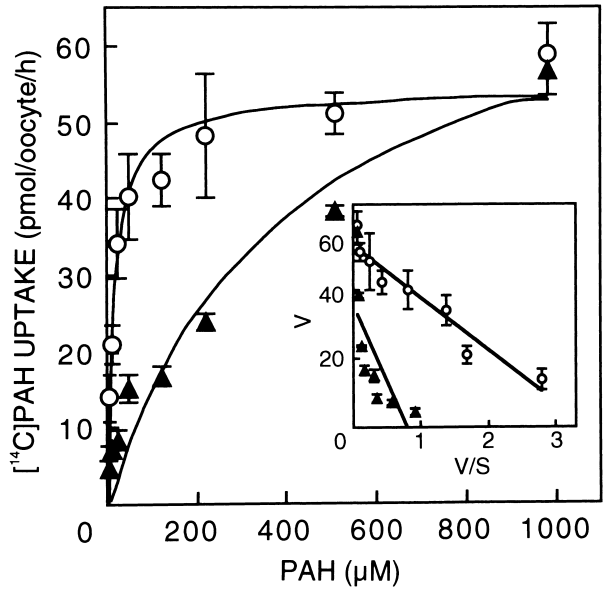

Fig. 3. Dose-dependent uptake of [^{14}C]*p*-aminohippurate by rOAT1-expressing oocytes with glibenclamide. [^{14}C]*p*-Aminohippurate uptake at various concentrations was measured for 1 h without (○) or with (▲) 10 μM glibenclamide. Inset shows Eadie–Hofstee plots of the data; *V*, uptake rate (pmol/oocyte/h); *S*, *p*-aminohippurate concentration (μM). Each point represents the mean ± S.E. of 5–7 oocytes.

3.4. Determination of the inhibition constant (K_i) values of chlorpropamide, glibenclamide, tolbutamide and nateglinide

Next, we calculated the K_i values of the four anti-diabetic drugs for rOAT1 using the equation: K_i value = IC_{50}/[1 + (concentration of *p*-aminohippurate/K_m value of *p*-aminohippurate)]. The values were estimated to be 39.5 μM for chlorpropamide, 1.6 μM for glibenclamide, 55.5 μM for tolbutamide and 9.2 μM for nateglinide.

3.5. Effect of chlorpropamide, glibenclamide, tolbutamide and nateglinide on [^{14}C]p-aminohippurate efflux via rOAT1

To clarify whether rOAT1 mediates translocation of the anti-diabetic drugs, we examined the trans-stimulation effects on [^{14}C]*p*-aminohippurate efflux via rOAT1. [^{14}C]*p*-Aminohippurate efflux was markedly accelerated in the presence of unlabeled *p*-aminohippurate as well as α-ketoglutarate. In contrast, there were no trans-stimulation effects of chlorpropamide, glibenclamide, tolbutamide and nateglinide, suggesting that these drugs could not be translocated via rOAT1 (Fig. 4). In addition, [^{3}H]glibenclamide uptake in rOAT1-expressing oocytes was similar to water-injected oocytes (10.99 ± 0.71 fmol/

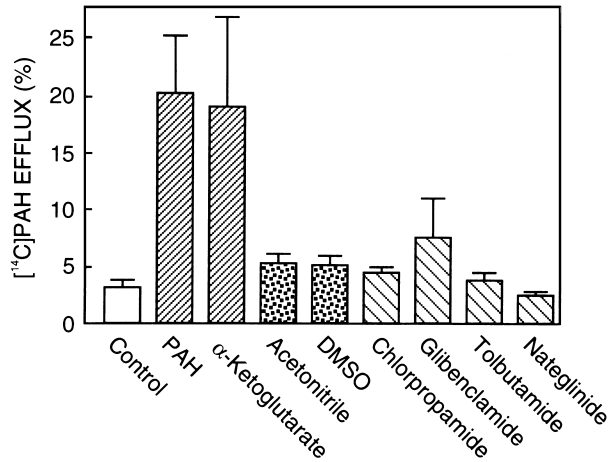

Fig. 4. [^{14}C] *p*-Aminohippurate efflux out of rOAT1-expressing oocytes in the presence of unlabeled *p*-aminohippurate, α-ketoglutarate, or various antidiabetics. Each finding was expressed as the ratio of the radioactivity of the incubation buffer to the sum of the radioactivities of the incubation buffer and oocytes. The concentration of the compounds to be tested was 1 mM except for glibenclamide at 50 μM, 0.5% acetonitrile or 1% DMSO. Each column represents the mean ± S.E. of 5–10 oocytes.

oocyte/3 h in rOAT1-expressing oocytes: 10.09 ± 0.25 fmol/oocyte/3 h in water-injected oocytes, mean ± S.E. of eight oocytes).

4. Discussion

In the present study, we investigated the interaction of sulfonylureas and nateglinide with rat renal organic anion transporter. Chlorpropamide, glibenclamide, tolbutamide and nateglinide are anionic compounds at physiological pH, having potent inhibitory effects on rOAT1-mediated *p*-aminohippurate uptake (Fig. 1). Among them, the inhibition by glibenclamide was the strongest (Fig. 2). In addition, a new oral anti-diabetic agent nateglinide, structurally unrelated to sulfonylurea, showed a high affinity for rOAT1.

Glibenclamide exhibits its pharmacological effect by inhibiting ATP-sensitive potassium channels in pancreatic β-cells (Babenko et al., 1998). The drug is reported to inhibit other ion channels such as the cystic fibrosis transmembrane conductance regulator (CFTR) or clinically important transporters (Sheppard and Welsh, 1992; Fükel and Petzinger, 1992; Huang et al., 1999; Sawada et al., 1999). Glibenclamide inhibited CFTR Cl^- currents at K_i value concentration of 20 μM and the inhibitory effect was not reversible (Sheppard and Welsh, 1992). Sawada et al. (1999) showed that glibenclamide inhibits rat peptide transporters PEPT1 and PEPT2 noncompetitively. The K_i values for PEPT1 and PEPT2 were 25 and 7.8 μM, respectively. Furthermore, carnitine transport in human kidney proximal tubular epithelial cells was inhibited by glibenclamide in a competitive fashion. The K_i value was estimated to be 3 μM (Huang et al., 1999). In rat hepatocytes, cholate and taurocholate uptake were inhibited by glibenclamide noncompetitively, and the K_i values were 9 and 75 μM, respectively (Fükel and Petzinger, 1992). Therefore, glibenclamide has been indicated to inhibit various types of membrane transporters and/or channels at relatively low concentrations. The binding affinity of glibenclamide for rOAT1 was the highest among the sulfonylureas tested. The K_i value of glibenclamide for rOAT1 was 1.6 μM. Melander et al. (1998) reported that the therapeutic efficacy of glibenclamide was obtained using a serum concentration ranging between 1 and 2 μM. Therefore, it is assumed that the half-life of drugs transported by rOAT1 will be prolonged and their side effects could be due to coadministration with glibenclamide.

Probenecid, a potent organic anion transport system inhibitor, prolongs the half-life of chlorpropamide (Petitpierre et al., 1972). Although glibenclamide and tolbutamide are eliminated by hepatic metabolism, the main route of chlorpropamide excretion appears to be mediated by renal tubular secretion via organic anion transporters (Hansen and Christensen, 1977). In the present study, we could not observe that rOAT1 mediates transport of chlorpropamide by trans-stimulation effect on *p*-aminohippurate efflux (Fig. 4). The failure of chlorpropamide to stimulate *p*-aminohippurate efflux might be due to the hydrophobicity of the drug, and/or to the minimal contribution of rOAT1 to renal secretion of the drug.

In conclusion, the present study demonstrates the first evidence that chlorpropamide, glibenclamide, tolbutamide and nateglinide interact with rOAT1, thereby inhibiting *p*-aminohippurate transport activity. Especially, glibenclamide shows a high binding affinity to rOAT1 with a K_i value of 1.6 μM. These findings suggested that sulfonylureas and nateglinide may cause rOAT1-mediated drug interactions by inhibiting renal tubular secretion of diverse anionic drugs.

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